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Responses to Chromatic and Luminance Contrast in Glaucoma: a Psychophysical and Electrophysiological Study

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Increasing anatomical evidence indicates that large retinal ganglion cells (M-cells) are preferentially damaged in primary open angle glaucoma (OAG), while the smaller ganglion cells (P-cells) are relatively spared. In 13 patients with defined OAG and modest visual field defects, we evaluated the responses to stimuli that are expected to involve primarily the function of the P-pathway and compared them with those of control subjects. The psychophysical contrast sensitivity (CS), the PERG and the VEPs were measured for red–green gratings of pure chromatic contrast, as well as yellow–black gratings of pure luminance contrast. As compared with controls, OAG patients had reduced CS for both luminance and chromatic contrast stimuli by about 6 dB. PERGs and VEPs to luminance stimuli were little affected, whereas those to chromatic stimuli were both reduced in amplitude and delayed. These results indicate that visual dysfunction in glaucoma is not selective for the M-pathway, and that responses to equiluminant colour-contrast stimuli may be of diagnostic value. © 1997 Published by Elsevier Science Ltd.

Chromatic stimuli Contrast sensitivity PERG VEP Glaucoma

INTRODUCTION

A considerable body of anatomical evidence indicates that large ganglion cells located in the retinal midperiphery are selectively damaged in human and experimental glaucoma, whereas the smaller and more central ganglion cells are relatively spared (Quigley *et al.*, 1987, 1988, 1989; Dandona *et al.*, 1991; Glovinsky *et al.*, 1993; see, however, Smith *et al.*, 1993; Johnson, 1994).

In primates, large and small ganglion cells have distinct retinofugal projections. Large ganglion cells (about 10% of the whole ganglion cell population) project to magnocellular (M) layers of the lateral geniculate nucleus (LGN) and then to lamina 4C-α of the striate visual cortex. Small ganglion cells (about 80% of the whole ganglion cell population) project to the parvocellular (P) laminae of the LGN and then to layer 4C-β of the striate cortex. The visual pathways from retinal ganglion cells that project to the P and M layers of the LGN (which we will call P and M ganglion cells) remain separate, at least to the level of the primary visual cortex.

The physiological properties of the two pathways differ considerably. The M cells have greater contrast sensitivity and contrast gain than P cells, although their response tends to saturate at high contrast (Kaplan & Shapley, 1982, 1986). Another difference is the temporal response to visual stimuli. The conduction velocity in the M pathway is much higher than in the P pathway (Dreher *et al.*, 1976; Marrocco, 1976). In addition, the response time course of M cells tends to be more transient, whereas that of P cells is more sustained (e.g., De Monasterio, 1978; Hicks *et al.*, 1983). In contrast, however, the great majority of cells in the P pathway display clear colour opponency, whereas most M cells are not colour coded (Wiesel & Hubel, 1966; De Monasterio & Gouras, 1975; Derrington *et al.*, 1984).

The finding of greater vulnerability of larger ganglion cells in glaucoma implies that a specific psychophysical/electrophysiological test for early detection should be designed to tap the activity of M ganglion cells. Indeed, several tests addressing visual functions subserved primarily by the M pathways (high frequency flicker sensitivity, motion detection, stereopsis) are reported to be abnormal in many glaucoma suspects with normal automated perimetry (Tyler, 1981; Schmeisser & Smith, 1989; Holopigian *et al.*, 1990; Silverman *et al.*, 1990; Bullimore *et al.*, 1993; Bassi & Galanis, 1991). However, several studies attempting to tap visual functions subserved primarily by the P-pathway have also shown these functions to be abnormal in early glaucoma. These include: colour perimetry (Hart *et al.*, 1990; Sample & Weinreb, 1990; see for review Felius, 1994), colour

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TABLE 1. Summary of clinical features of patients

Patient	Sex	Age (yr)	Time elapsed* (months)	Visual acuity	Pupil diam. (mm)	IOP (mmHg)	MD† (dB)	CPSD‡	GHT§
1	M	68	3	10/10	3.5	14	−10.88	7.00	2
2	F	54	12	10/10	3.0	16	−1.67	3.57	1
3	F	52	8	10/10	3.5	16	−1.77	3.99	2
4	F	80	10	8/10	3.5	14	−0.55	0.00	0
5	F	66	1	10/10	4.0	16	−15.66	9.02	2
6	F	71	7	9/10	4.5	14	−0.33	2.19	1
7	F	74	1	10/10	4.0	16	−4.39	2.55	2
8	F	73	6	10/10	4.0	15	−0.45	1.00	0
9	F	65	8	10/10	3.5	14	−0.36	1.52	1
10	F	79	6	8/10	2.5	21	−3.60	2.36	1
11	F	64	3	10/10	4.0	10	−4.60	4.12	2
12	F	55	1	10/10	3.5	14	−6.51	0.00	1
13	M	80	2	10/10	3.5	16	−4.50	6.48	2

*Time from the first diagnosis.
†Mean deviation.
‡Corrected pattern standard deviation.
§Glaucoma hemifield test: 0 = within normal limits, 1 = borderline, 2 = outside normal limits.

discrimination sensitivity (e.g., Lakowski & Drance, 1979; Adams *et al.*, 1987; Gündüz *et al.*, 1988; Greenstein *et al.*, 1993), colour PERG (Korth *et al.*, 1993) and colour VEPs (Korth *et al.*, 1994; Seliger *et al.*, 1995).

Clearly, the above results suggest that the size-dependent ganglion cell loss in glaucoma cannot be explained solely on the basis of a selective vulnerability of M cells. Glovinsky *et al.* (1991) have proposed that loss of larger P cells probably accounts for visual dysfunctions subserved by the P-pathway.

However, the anatomical picture in glaucoma does not necessarily correlate with the visual dysfunction. Morphologically intact ganglion cells may well present a variable degree of dysfunction which may manifest itself in alteration of some psychophysical and/or electrophysiological test. Overall, the question remains as to the differential vulnerability of the M- and P-pathway in glaucoma.

In the present study we have evaluated, in the same subjects, the psychophysical contrast sensitivity and recorded the PERG and the VEPs in response to achromatic, luminance modulated gratings and to chromatic (red–green) equiluminant gratings. For the electrophysiological recordings, the stimuli were either modulated at various temporal frequencies, luminance or chromatic contrast, or transiently reversed in luminance or chromatic contrast. Data have been obtained from a group of patients with open angle glaucoma (OAG) in relatively early stages, as well as in age-matched controls. It will be shown that the patients differ from controls in different ways according to the type of test and the type of stimulus used (achromatic or chromatic-equiluminant gratings, sinusoidally or transiently modulated, etc.). The findings are discussed in terms of involvement of the P and M pathways and indicate that the visual dysfunction is not limited to the M pathway.

METHODS

Subjects

Thirteen patients attending the eye clinic at the University of Pisa were included in the study. Eleven were females and two were males; their ages ranged from 52 to 80 yr (mean 67.0, SD 11.3). Their visual acuity was better than 0.8. They were diagnosed as having bilateral open angle glaucoma (OAG) on the basis of an established history of elevated intraocular pressure and abnormality at either Humphrey 30-2 error scores (MD, CPSD, GHT) or glaucomatous disk cupping (c/d). All patients were being treated with timolol maleate, and none of them underwent antiglaucomatous surgery. According to Sponsel *et al.* (1995), 10/13 patients were in an early stage of disease (MD greater than −6 db), 2/13 were in a moderate stage (MD between −6 and −12 db), and one patient was in a relatively advanced stage (MD worse than −12 db). Clinical details of patients are reported in Table 1. Ten normal subjects (seven females, three males) with visual acuity ≥ 1.0, age-matched with patients (mean age 63 yr, SD 9), also participated in the study. All subjects were free from congenital colour defects and had no or small refractive errors, which were fully corrected for the viewing distances. No artificial pupils or dilation agents were used. All experiments followed the tenets of the Declaration of Helsinki. Informed consent was obtained after the aims and the experimental procedures were fully explained. Psychophysical and electrophysiological responses have been obtained from both eyes. In this study we report data from one eye only, which has been randomly chosen in both normal controls and patients.

Visual stimuli

The stimuli were horizontal sinusoidal gratings, modulated in either luminance or chromaticity, generated by framestore (Cambridge Research VSG/2, U.K. and

displayed on the face of a colour monitor at a frame rate of 120 Hz, 512 lines per frame, 14 bits per colour per pixel (Barco CCID 7751, Belgium) suitably linearized by gamma correction (Minolta Chromameter CS 100). The peak spectral response for the red phosphor was at 628 nm (CIE co-ordinates: $x = 0.618$, $y = 0.351$) and that of the green phosphor 531 nm (CIE co-ordinates: $x = 0.286$, $y = 0.601$). Stimuli were obtained by combining red and green gratings of identical contrast and luminance. Luminance (yellow–black) stimuli were made by summing the red and green gratings in phase, and the chromatic stimuli (red–green) by summing them in counterphase. Following the procedure introduced by Mullen (1985), the relative contribution of the red and green was varied by modulating the red and green guns. The red and green components had the same contrast, defined as $[C = (L\text{max} - L\text{min})/(L\text{max} + L\text{min})]$, where Lmax and Lmin are the peak and through luminance values. The relative luminance of the two components was variable: the colour ratio $[r = \%R(R + G)]$ of red to total luminance varied from 0 to 100, where $r = 0\%$ defined a green–black pattern, $r = 100\%$ a red–black pattern, and intermediate values a red–green chromatic pattern. To minimize the response from short-wavelength cones, the patterns were viewed through yellow filters (Kodak Wratten W16) that heavily attenuated wavelengths below 500 nm. Viewed through the filter, the CIE co-ordinates for the red were $x = 0.647$, $y = 0.351$ and for the green $x = 0.392$, $y = 0.606$. The response of the long (L) and medium (M) wavelength cones was calculated from the CIE values of the display phosphors and human cone fundamentals (Smith & Pokorny, 1975). Data showed that under the above experimental conditions L cones and M cones do not modulate at $r = 43\%$ and $r = 68\%$, respectively (points of silent substitution: Estevez & Spekreijse, 1982). For $r = 50\%$, the response of L and M cones is equal and opposite. The equiluminant point for normal adult observers is near this ratio (Fiorentini *et al.*, 1966). Further details on the evaluation of the silent substitution points for the red and green, and evaluation of the cone contrast can be found elsewhere (Morrone *et al.*, 1993, 1994a).

The visible screen was 26 cm wide and 24 cm high, subtending an area of 53×49 deg when viewed from 28 cm (electrophysiologic experiments) and 15×14 deg from 100 cm (psychophysical experiments). Mean luminance was 17 cd/m^2, producing a retinal illuminance of about 175 Td when viewed through natural pupils, measured to be about 3.6 mm diameter, on average, in both control subjects and patients.

Psychophysical procedure

In all subjects the equiluminant point was established monocularly, by measuring contrast threshold (method of ascending limits) for red–green gratings (1 c/deg), sinusoidally reversed in contrast at 5 Hz (10 rev/sec), as a function of the proportion of red in the red–green mixture (see previous section). For each color ratio the contrast sensitivity of the red–green grating was defined as the reciprocal of contrast (same for the red and green components) at detection threshold. For every subject, the colour ratio corresponding to the lowest contrast sensitivity has been taken as the equiluminant point (see Results). That value was used for all chromatic and luminance stimuli employed in electrophysiological recordings. Previous controls in normal subjects (not shown in figures) demonstrated that the equiluminant point does not change significantly in the frequency range 2–20 Hz.

Electrophysiological techniques

After the equiluminant point had been established, electrophysiological recordings were made with stimuli (0.3 c/deg spatial frequency) of constant mean luminance, spatially modulated in either pure luminance contrast (yellow–black) or pure chromaticity (equiluminant red–green), reversed sinusoidally in contrast at frequencies ranging from 2 to 24 Hz (for steady-state responses) and at 1 Hz (square-wave) for transient responses. PERGs were recorded monocularly by means of Ag/AgCl superficial cup electrodes, 9 mm diameter, positioned over the lower eyelid. An equal electrode, positioned over the eyelid of the contralateral, patched eye, served as reference. As the recording protocol was extensive, this electrode placement represented a good compromise between signal-to-noise ratio and signal stability. Discussion on the PERG by skin electrodes and its relationship with the PERG by corneal electrodes can be found elsewhere (e.g., Porciatti, 1987; Hawlina & Konec, 1992; Porciatti & Falsini, 1993). VEPs were recorded simultaneously using the same type of electrodes, placed 2 cm above the inion (active) and at the right mastoid (reference). The common ground for all recordings was located on the forehead. PERG and VEP signals were amplified (PERG 100,000 fold; VEP 50,000 fold), band-pass filtered between 1 and 100 Hz (6 dB/oct), digitized at 1024 Hz with 12 bit resolution and averaged on-line by a PC. The computer rejected single sweeps over a threshold voltage (4 V) to minimize gross potential changes induced by eye blinks, ocular movements, or other biological activities. The PC averaged the PERG and VEP in synchrony with stimulus periodicity and performed a discrete Fourier transform (DFT) to evaluate the amplitude and phase of the dominant response component (second harmonic). As averaging was performed over one integer stimulus period, DFT spectra contained only the harmonics of stimulus frequency without leakage to neighbouring frequency bands, and windowing was not necessary. Second harmonic amplitude and phase were also calculated separately for partial sums (40-sum packets) of the total average (at least 280 sums), from which the standard error of the amplitude and phase estimates were derived to test response reliability (Porciatti *et al.*, 1992). The program also averaged the signals asynchronously at 1.1 times the temporal frequency of the stimulus and evaluated the second harmonic component of this waveform to provide an estimate of background noise. Transient PERGs were

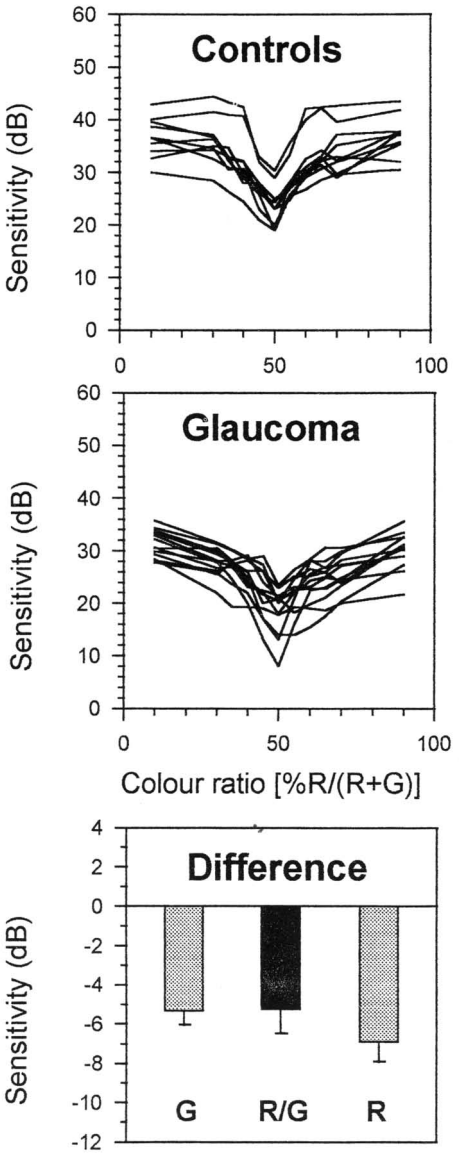

FIGURE 1. Contrast sensitivity for detection of red–green gratings of various colour ratios modulated in counterphase at 5 Hz, measured in control observers and in glaucoma patients. Sensitivity is expressed in attenuation of maximum contrast at detection threshold. The colour ratio at which the sensitivity is lowest (equiluminant point) is of the order of 50% both in controls and patients. Glaucoma eyes display an overall loss of sensitivity. The bottom panel shows that the average (±SEM) sensitivity loss (difference from the mean of normal controls) in glaucoma eyes is similar for equiluminant gratings and luminance gratings (either green–black or red–black)

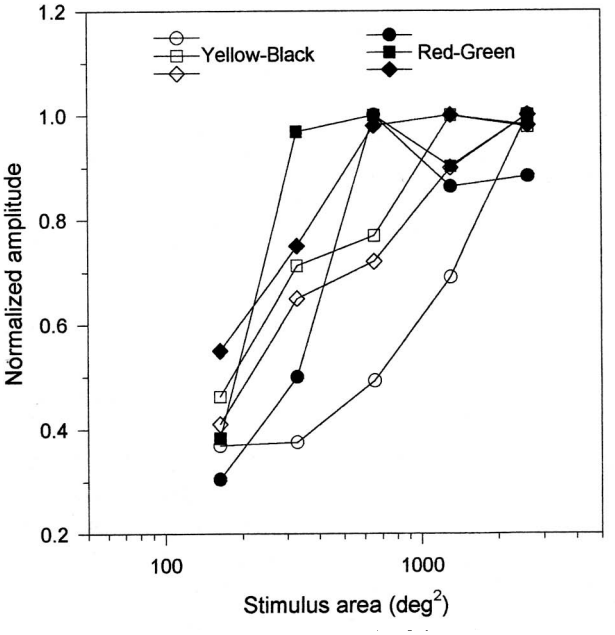

FIGURE 2. Amplitude (normalized to maximum) of the steady-state PERG as a function of the area of stimulation for both equiluminant red–green gratings (filled symbols) and luminance yellow–black gratings. Different symbols refer to different observers. Note that the chromatic PERG, as compared to the luminance PERG, saturates for considerably smaller stimulus sizes. Spatial frequency: 0.3 c/deg, contrast: 90%, temporal frequency: 5 Hz.

smoothed off-line by running average over 10 points to remove most high-frequency noise arising from eyelid muscle activity, thereby allowing a more precise evaluation of amplitude and latency measurements.

RESULTS

Psychophysics

Figure 1 shows how the CS for red–green gratings sinusoidally modulated in counterphase at 5 Hz change as a function of the colour ratio. For both control and OAG eyes there is a clear minimum at colour ratio around 50% and the function is symmetrical about that point. The colour ratio corresponding to the lowest contrast sensitivity has been taken as the equiluminant point. In Fig. 1 three major aspects are readily apparent: (1) the equiluminant point is similar in control and OAG eyes; (2) there is a drop of sensitivity in patients; and (3) the threshold elevation is rather uniform across colour ratio. Individual sensitivity differences from the normal average have been evaluated for stimuli of pure chromatic contrast (at the equiluminant point) and high luminance contrast (green–black and red–black at colour ratios 10% and 90%, respectively). The results are reported in the bottom panel of Fig. 1. For green–black, red–green and red–black patterns there is a significant sensitivity loss in patients of the order of 6 dB (*t*-tests for rejecting the null hypothesis: $P < 0.01$). The sensitivity loss is not significantly different for colour contrast and luminance contrast (Repeated measures ANOVA:$F(2,38) = 1.3$, $P = 0.29$).

Electrophysiology: steady-state responses

Examples of steady-state PERG and VEPs, recorded simultaneously in response to either equiluminant red–green gratings or yellow–black stimuli of the same contrast (90%) and colour ratio (50%), modulated in counterphase at different temporal frequencies have been reported elsewhere (Morrone *et al.*, 1994a; Porciatti & Sartucci, 1996). As all responses have an approximately sinusoidal waveform with strong modulation at twice the stimulus frequency, overall amplitude and latency are

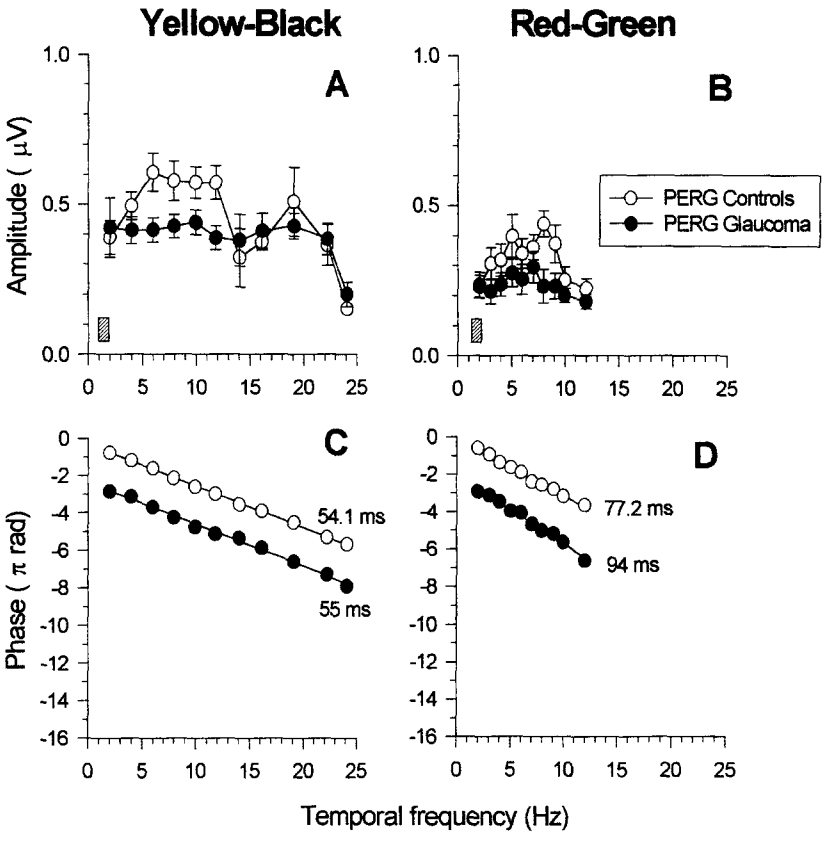

FIGURE 3. Group averages (±SEM) of the second harmonic amplitude (A, B) and phase (C, D) of the steady state PERG in response to yellow–black luminance gratings (A, C) and red–green equiluminant gratings (C, D) for control subjects (open symbols) and glaucoma patients (filled symbols), as a function of stimulus temporal frequency. Note that in glaucoma both the luminance and chromatic PERG are somewhat reduced in amplitude. The chromatic PERG is also delayed in apparent latency.

well represented by second harmonic amplitude and phase.

In this study we used a stimulus of low spatial frequency (0.3 c/deg) for both colour and luminance. At this spatial frequency the luminance and colour PERG have approximately the same amplitude (Morrone *et al.*, 1994a). The field size had a very large area (53 × 49 deg) to include a significant number of cycles in the stimulus. A large area was also necessary to tap significant activity from extrafoveal retina, known to be particularly vulnerable in OAG. As shown in Fig. 2 for three different normal subjects, the amplitude of both chromatic (filled symbols) and luminance PERG (open symbols) increases as a function of stimulus size and tends to saturate for the largest sizes. However, the chromatic PERG saturates for considerably smaller stimulus sizes (300–600 deg^2) as compared with the luminance PERG (1500 deg^2 or beyond). The effect is statistically significant [two-way ANOVA: interaction effect stimulus by area $F(4,29) = 4.1$, $P = 0.04$]. This experiment indicates that the chromatic and luminance stimuli sample the activity of generators with different retinal distributions.

The effect of temporal frequency on the PERG to yellow–black luminance and red–green chromatic stimuli is summarized in Fig. 3. Figure 3(A) shows the amplitude spectra (averaged over subjects) and Fig. 3(B) shows the corresponding phase spectra. The dashed bar in Figs. 3(A) and (B) indicates the noise range (averaged over temporal frequency), which had the same value for both controls and patients. As shown in Fig. 3(A), the luminance PERG of control subjects is temporally tuned in amplitude, with a broad maximum between 6 and 12 Hz, a secondary maximum at around 20 Hz and a high temporal frequency cut-off between 25 and 30 Hz. On average, the PERG amplitude of OAG eyes is reduced by about 13% as compared to control eyes [two-way ANOVA: $F(1,252) = 6.6$, $P = 0.01$]. Amplitude reduction appears more marked for the low (6–12 Hz) than for the high (20–22 Hz) temporal frequency range. However, the interaction effect between eye group and temporal frequency is not significant [$F(10,252) = 1.7$, $P = 0.07$]. As compared with the luminance PERG, the chromatic PERG has a lower amplitude and is recordable over a shorter range of temporal frequencies. In OAG patients the chromatic PERG is reduced by about 12% on average [two-way ANOVA: $F(1,252) = 6.6$, $P = 0.01$]. The interaction effect between eye group and temporal frequency is not significant [$F(10,252) = 1.5$, $P = 0.11$].

As shown in Fig. 3(C) and Fig. 3(D), the PERG phase lags progressively with increasing temporal frequency for all eye groups. The response phase bears a relationship to the response latency. However, it is not possible to have an absolute estimate of latency from a single steady-state response, since there is an infinite set of phases separated

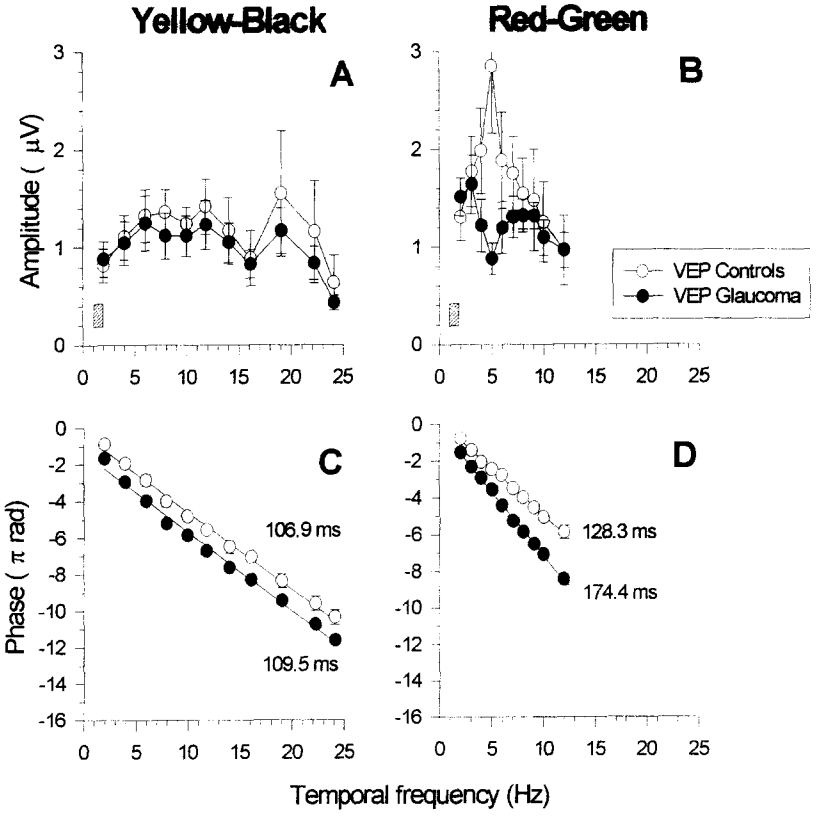

FIGURE 4. Group averages (±SEM) of the second harmonic amplitude (A, B) and phase (C, D) of the steady-state VEPs in response to yellow–black luminance gratings (A, C) and red–green equiluminant gratings (C, D) for control subjects (open symbols) and glaucoma patients (filled symbols), as a function of stimulus temporal frequency. Note in glaucoma that the luminance VEPs are unaltered in amplitude and apparent latency. The chromatic VEPs are selectively reduced in amplitude at about 5 Hz and much delayed in apparent latency.

by 2π rad. Response latency (apparent latency) can be evaluated by measuring phase as a function of temporal frequency, and estimating the slope of the curve according to the formula [Apparent latency (s) = Phase slope (π rad/Hz) /4($2 \times 2\,\pi$ rad, period of the second harmonic)] (Regan, 1966; Spekreijse *et al.*, 1977; Porciatti *et al.*, 1992; Morrone *et al.*, 1994a). It should be noted in Figs. 3(C) and (D) that in control subjects the average slope (evaluated by fitting data over all observers) of the chromatic PERG is steeper than that of the luminance PERG, corresponding to a delay in apparent latency of about 23 msec. Statistics (made on apparent latencies evaluated from phase slopes of individual subjects) showed that the difference in apparent latency is significant [$t(9) = 9.5$, $P < 0.001$]. In OAG eyes, the average apparent latency of the luminance PERG is of the same order as that of control eyes (55 vs 54.1 msec), whereas that of the chromatic PERG is delayed [statistics on individual apparent latencies $t(21) = 2.53$, $P = 0.019$] by about 17 msec as compared with controls (94 vs 77.2 msec).

Results obtained by recording steady-state VEPs to yellow–black or red–green stimuli of different temporal frequencies are summarized in Fig. 4. As previously reported for luminance contrast gratings (Porciatti *et al.*, 1992; Porciatti & Sartucci, 1996), the VEP second harmonic is temporally tuned in amplitude in control subjects [Fig. 4(A)], with a maximum at 6–8 Hz, a secondary maximum around 20 Hz and a cut-off between 25 and 30 Hz. In OAG eyes, there is a tendency to an overall reduction in amplitude, but the changes are small and not significantly different from controls [two-way ANOVA, eye group: $F(1,252) = 1.19$, $P = 0.27$; interaction eye group by temporal frequency: $F(10,252) = 0.07$, $P = 1$]. The temporal function of VEPs to red–green equiluminant gratings differs from that of the luminance VEPs in control subjects. As shown in Fig. 4(B), the amplitude maximum occurs at a lower frequency (5 Hz). For higher frequencies there is a steep fall-off in amplitude and no reliable responses can be recorded at 15 Hz or higher (in agreement with previous results: Fiorentini *et al.*, 1991; Morrone *et al.*, 1993). In OAG patients, the chromatic VEP is reduced by about 26% on average [two-way ANOVA: $F(1,229) = 10.8$, $P = 0.012$). Interestingly, amplitude reduction is rather specific for frequencies at or around the peak region, whereas for higher and lower frequencies the VEP amplitude is in the range of that of controls. Consequently, the form of the VEP temporal tuning function in patients is significantly different from that of controls [interaction effect between eye group and temporal frequency: $F(9,229) = 2.2$, $P = 0.023$]. As for the PERG phase, the VEP phase lags progressively with increasing temporal frequency for all eye groups and conditions. The functions could be well fit

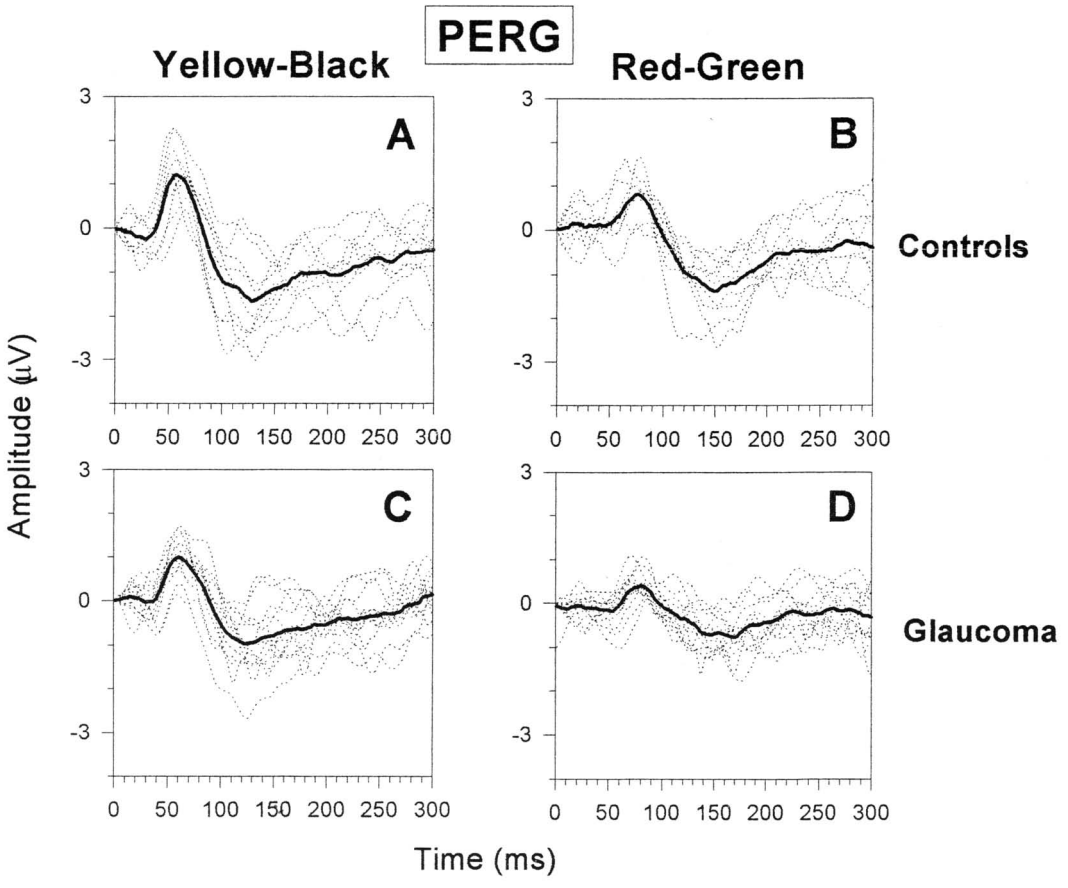

FIGURE 5. Transient PERGs in response to either yellow–black luminance gratings (A, C) or red–green equiluminant gratings (B, D) recorded in control eyes (A, B) and glaucoma eyes (C, D). For each panel, individual waveforms are displayed as dotted lines, while the bold lines represent the grand means. Note in glaucoma eyes that the negative component of chromatic PERG is severely reduced in amplitude.

by a linear regression line. It can be seen in Figs. 3(C) and (D) that in control subjects the average apparent latency for the chromatic VEP, as compared with the luminance VEP, is longer by about 22 msec [128.3 vs 106.8 msec; statistics on individual apparent latencies: $t(9) = 3.68$, $P = 0.005$]. In OAG eyes, the average apparent latency for the luminance VEP is of the same order as that of control eyes (109.5 vs 106.8 msec), whereas that of the chromatic PERG is considerably delayed by about 47 msec [174.4 vs 128.3 msec: statistics on individual apparent latencies: $t(21) = 4.34$, $P < 0.001$].

Electrophysiology: transient responses

Transient PERGs of all subjects are summarized in Fig. 5. The left-hand panels show the waveforms of the luminance PERG and the right-hand panels those of the colour PERG. The bold line waveforms represent the grand mean of individual waveforms (dotted lines). Inspection of grand averages indicates that in control subjects the colour PERG [Fig. 5(B)] has a waveform similar to that of the luminance PERG [Fig. 5(A)]. However, it is slightly smaller in amplitude and delayed in the latency of the positive peak (P_{80} vs P_{60}). In patients, the average peak-to-trough amplitude is somewhat reduced for both kinds of stimuli. Amplitude reduction appears larger for the negative component of the colour PERG.

Statistics has been performed on amplitude and latency values of individual waveforms. In control subjects, the positive peak latency of the colour PERG is delayed, on average, by 20 msec with respect to the luminance PERG [$t(9) = 5.8$, $P < 0.0001$]. This delay is similar to that found in the steady-state responses (see above). Amplitude losses in patients have been evaluated separately for the positive and negative components, and the results of statistical comparisons are summarized in Table 2. The

TABLE 2. Amplitude reductions of PERG components in glaucoma patients

	Luminance PERG	Colour PERG
Positive component	−22%; $t(21) = 1.89$, $P = 0.07$	−14%; $t(21) = 0.9$, $P = 0.37$
Negative component	−23%; $t(21) = 1.98$, $P = 0.06$	−40%; $t(21) = 3.81$, $P = 0.001$

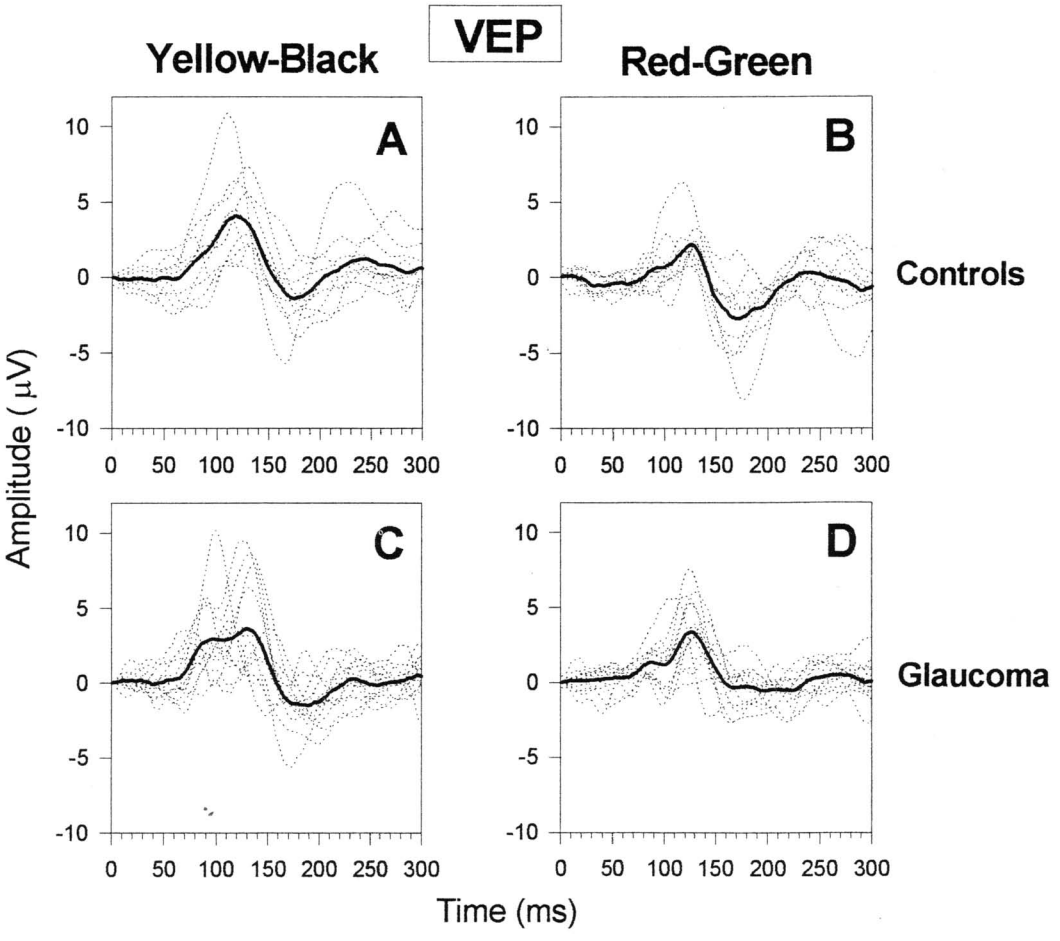

FIGURE 6. Transient VEPs in response to either yellow–black luminance gratings (A, C) or red–green equiluminant gratings (B, D) recorded in control eyes (A, B) and glaucoma eyes (C, D). For each panel, individual waveforms are displayed as dotted lines, while the bold lines represent the grand means.

only significant reduction is for the negative component of the colour transient PERG.

Figure 6 displays transient VEPs recorded simultaneously to PERGs presented in Fig. 5. In control subjects, the waveform of VEPs to yellow–black gratings [Fig. 6(A)] consists mainly of a broad positive peak with an average latency of about 120 msec. The time to peak of this positive wave and the amplitude from the preceding negativity were taken as representative of latency and amplitude values of individual responses. The average colour VEP [Fig. 6(B)] displays a positive–negative complex. However, the positive peak is less consistent among subjects and the most reliable wave is a negative one, peaking at 170 msec on average. This agrees with previous reports showing that the VEPs to red–green chromatic contrast consists mainly of a late negative wave (Murray *et al.*, 1987; Berninger *et al.*, 1989; Fiorentini *et al.*, 1991, 1996c). The time to peak of this negative wave and the amplitude from the preceding positivity were taken as representative of latency and amplitude values of individual responses (see Fig. 7). In patients, the grand averages of the luminance [Fig. 6(C)] and colour [Fig. 6(D)] transient VEPs appear somewhat different from controls. In particular, the positive peak of luminance VEPs tends to split up, whereas the negative component of colour VEPs tends to be reduced in amplitude and delayed in latency. However, individual VEPs of patients, compared with controls, are more variable in waveform and jittered in latency, so that the grand averages probably overemphasize amplitude losses. Statistics (*t*-tests) performed on average amplitude and latency values of individual waveforms do not reveal significant differences between controls and patients for either luminance or colour VEPs.

Figure 7(A–D) summarizes individual PERG and VEP data obtained from both transient (upper panels) and steady state responses (lower panels) as amplitude vs latency scatterplots. For transient responses, data represent the amplitude and peak latency of the main component (see above). For steady-state responses the amplitude data represent the second harmonic averaged across temporal frequency, whereas apparent latency data have been evaluated from the slope of the phase lag with temporal frequency. Dotted and dashed lines indicate the confidence limits of the normal range for responses to luminance and colour, respectively. Owing to the larger scatter of amplitude and latency values in patients as compared with controls, some measures exceed the limits

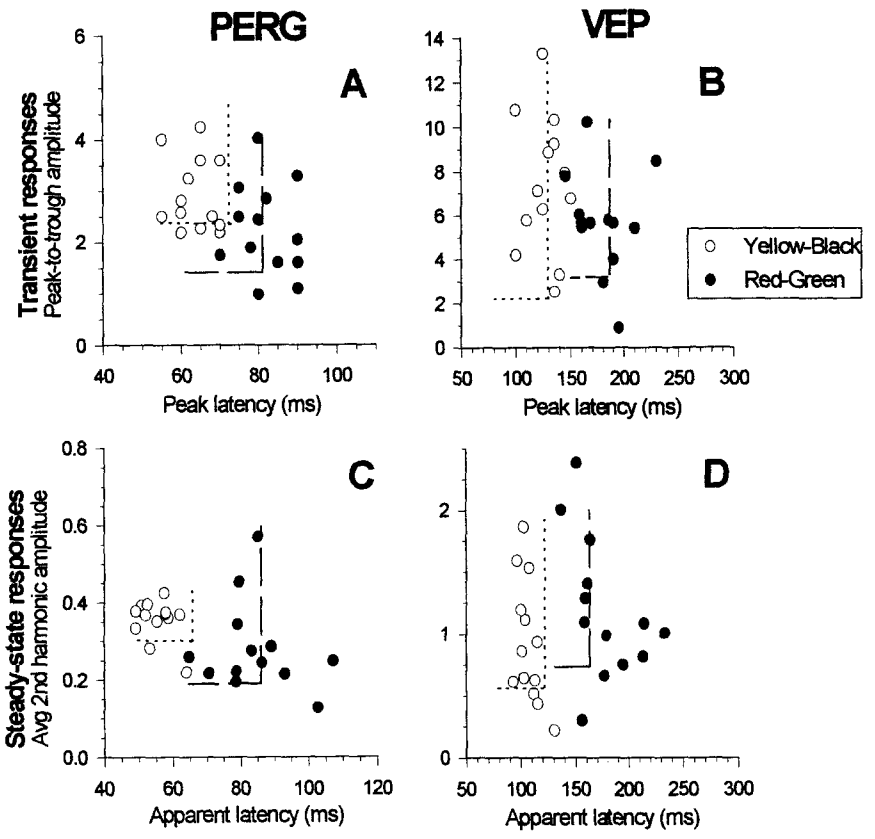

FIGURE 7. Amplitude vs latency scatterplots of transient (A, B) and steady state (C, D) PERGs (A, C) and VEPs (B, D) measured in individual eyes of glaucoma patients for either yellow–black luminance gratings (open circles) or red–green equiluminant gratings (filled circles). For transient responses (A, B), the peak latency and the peak-to-trough amplitude refer to data evaluated from the representative response component (see results for details). For steady-state responses (C, D) the average second harmonic amplitudes and apparent latencies refer to data evaluated from the whole range of temporal frequencies tested (see results for details). Dotted and dashed lines represent the limits of the normal range.

of the normal range. Overall, responses to colour are more frequently altered than the response to luminance. In particular, 6/13 transient—and 5/13 steady-state colour PERGs display values beyond the normal range, whereas luminance PERGs do in 4/13 transient—and 2/13 steady state responses. Colour VEPs are abnormal in 6/13 (transient) and 7/13 cases (steady state). Luminance VEPs are altered in 6/13 (transient) and 3/13 (steady state) cases. It is worth noting that latency abnormalities are more frequent in the colour responses than in the luminance responses.

Correlation with clinical data

An interesting question is whether relative changes of colour and luminance responses in glaucoma depend on the clinical stage of the disease. If generators of colour and luminance responses were differently vulnerable in glaucoma, one should expect a change of the colour-to-luminance response ratio with advancing disease. In Fig. 8, colour-to-luminance amplitude ratios and colour-minus-luminance latency differences of individual patients are plotted against corresponding values of the central visual field sensitivity (Humphrey 30-2 mean deviation). Upper panels represent data of transient PERGs and VEPs and lower panels represent data of steady-state PERGs and VEPs. A relatively larger loss of luminance responses than colour responses with advancing visual field damage results in a negative regression for amplitude ratio and a positive regression for latency difference. Inspection of Fig. 8 indicates that there is no systematic trend to a differential impairment of colour and luminance responses with advancing visual field damage. Statistical correlations (Pearson) were not significant for all measures. Comparable results have been obtained by correlating visual field losses with colour/luminance relative deviations from the normal average (amplitude losses and latency delays). Statistical correlations (Pearson or Spearman) between electrophysiological losses and other clinical parameters (CPSD, GHT) were also not significant.

DISCUSSION

Under most circumstances it is difficult to stimulate selectively the magno- or the parvo-cellular pathway (e.g., Logothetis *et al.*, 1990). However, there are particular stimulus conditions which are likely to favour the responses of one or the other cell population. That primarily the P-pathway is responsible for the threshold responses to red–green patterns with pure colour contrast

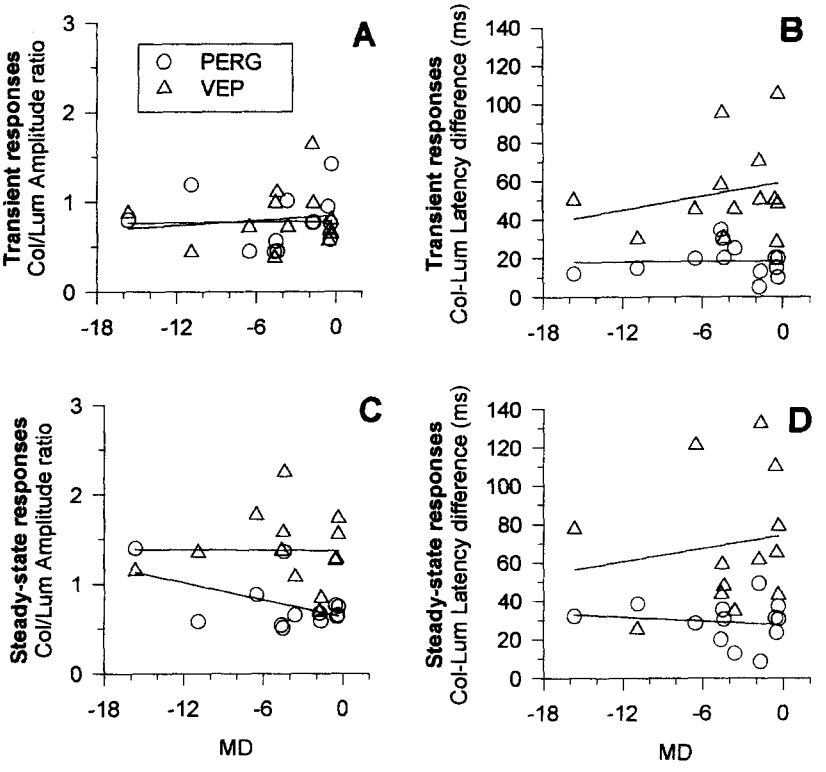

FIGURE 8. Relative changes of colour to luminance amplitude ratios (A, C) and latency differences (B, D) as a function of visual field loss (mean deviation) for transient (A, B) and steady state (C, D) PERG (open circles) and VEP (open triangles). Note that amplitude ratios and latency differences are fairly invariant with visual field loss.

is suggested by experiments in monkeys, showing that colour contrast sensitivity vanishes (while luminance contrast sensitivity may remain intact) in animals with selective lesions of the P cells in the lateral geniculate nucleus. Selective lesions in the magnocellular layers of the LGN, on the contrary, leave the colour contrast sensitivity intact and impair the sensitivity for luminance contrast more or less, depending on the spatial and temporal frequency (Merigan *et al.*, 1991; Merigan & Maunsell, 1993). In the present study we have compared, in a small group of patients with glaucoma in relatively early stages, the responses to stimuli designed to favour the activity of either the magno- or the parvo-cellular system.

In patients, the contrast sensitivity for equiluminant red–green patterns has been found to be impaired by significant amounts as compared to controls. The sensitivity to luminance contrast is also impaired to a similar extent. This loss of sensitivity for both luminance contrast and chromatic contrast is not consistent with a selective dysfunction of the M pathway. Rather, it is consistent with an involvement of both the P and M pathway. An alternative hypothesis is that dysfunction is at a level peripheral to the origin of the two separate pathways. However, although involvement of the outer retina has been documented in advanced glaucoma (e.g., Henkes, 1951; Holopigian *et al.*, 1990), this does not seem to be the case for patients with relatively early stages of glaucoma (Kendell *et al.*, 1995).

While the psychophysical findings provide information on the elevation of contrast threshold, the electrophysiological experiments evaluate the responses of the visual pathway to stimuli of high contrast. Also, for these responses the patients differ from the controls, primarily for the equiluminant chromatic patterns. Overall, the luminance PERG and VEP were only slightly affected in amplitude and unchanged in latency, while the chromatic PERG and VEP might be both reduced in amplitude and delayed. In particular, the chromatic steady-state PERG was delayed, and the negative component of the chromatic transient PERG was depressed. The chromatic steady-state VEP were delayed and reduced in amplitude. Interestingly, amplitude reduction was confined to a narrow band of temporal frequency. This implies higher vulnerability in glaucoma for a subset of generators of response to colour. Amplitude reduction for the steady-state VEPs to chromatic, but not luminance, stimuli has been also reported in optic neuritis (Porciatti & Sartucci, 1996). However, amplitude reduction was rather uniform as a function of temporal frequency.

The selective alteration of the apparent latency of the steady-state chromatic PERG in glaucoma may be interpreted as resulting from a longer integration time of the generators of this response, that are known to be located in the inner retinal layers (Maffei & Fiorentini, 1981; Baker *et al.*, 1988; Morrone *et al.*, 1994b). The remarkable increase in latency of the steady-state chromatic VEPs indicates an additional source of delay

at postretinal level. No significant delay of the luminance PERG and VEPs has been observed in our patient group. Again, these findings are difficult to reconcile with a predominant dysfunction of the M pathway. If the activity of mainly the M population were compromised by glaucoma, one would expect the PERG and the VEP to be affected in response to luminance contrast modulation, at least by a comparable amount as for colour contrast modulation. The latency of the PERG and VEPs for black and white checkerboards with luminance contrast has been sometimes reported to be prolonged to a small extent in patients with early glaucoma (Marx *et al.*, 1987; Price *et al.*, 1988; Sokol *et al.*, 1981). Different experimental conditions may explain the apparent discrepancy with the present results: the spatial frequency spectrum of checkerboard stimuli contains a range of high spatial frequencies that probably favour a larger contribution of the P pathway (e.g., Merigan *et al.*, 1991). Our luminance modulated stimuli of low spatial frequency are likely to involve the M system to a greater extent, especially at medium to high temporal frequencies. It is interesting, therefore, that for these stimuli the latency of the responses is not altered in patients.

The anatomical evidence for a loss of large ganglion cells in glaucoma is suggestive of a dysfunction of the magnocellular pathway. Our findings do not contradict this possibility, but point to an additional dysfunction of the parvocellular pathway. The relative dysfunction of the parvocellular and magnocellular pathways appears to be rather independent of the extent of visual field loss. We cannot exclude the possibility that our patients were already beyond a disease stage at which a preferred damage of the magnocellular system had occurred. In order to test this hypothesis, the present approach should be extended to glaucoma suspects with early optic disk changes. However, a recent comprehensive review of psychophysical measures performed in glaucoma-suspect eyes (Sample *et al.*, 1994) indicates abnormality of a variety of tests designed to isolate either the magnocellular or the parvocellular pathway.

In the present study, the abnormalities of colour responses imply an impairment in the red–green neural channel which does not result from an imbalance of red and green, since the equiluminant point is not altered. The dysfunction in the red–green channel adds to the previous evidence of an impairment of the blue–yellow channel in glaucoma (see for review Felius, 1994; Sample *et al.*, 1994; Korth *et al.*, 1993, 1994). It is commonly assumed that the blue–yellow channel is altered early in glaucoma. However, it is difficult to state whether the blue–yellow channel and the red–green channel are differently altered, since comparisons of blue–yellow losses with red–green losses in the same subjects are scanty. Available psychophysical (e.g., Greenstein *et al.*, 1993) and electrophysiological (Korth & Horn, 1990) evidence suggests rather comparable losses of blue–yellow and red–green. In the present study, blue–yellow could not be tested since at the luminance levels available in our set-up, the PERG is not reliable enough.

CONCLUSIONS

Our findings show that glaucoma in relatively early stages does not result merely in a selective deficit of the M system: responses ascribable to the P system are also affected. However, caution is required when speculating on the relative contribution of M and P neural populations to the visual loss of glaucoma patients. And indeed, one may arrive at different conclusions on the possible involvement of the two systems depending upon the type of data collected (psychophysical contrast threshold measurements vs electrophysiological responses to high contrast patterns) and the level of the visual pathway tested (PERG vs VEP).

For clinical applications, aiming either at improving the knowledge of the pathophysiological mechanisms of glaucoma, or at designing a sensitive test for early diagnosis, it seems advisable to rely upon a battery of different tests.

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